

# Use of pyrosequencing to investigate the inhibitory effect of dietary phytonutrients on the proliferation of *Eimeria maxima* in broilers



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## Introduction

- Avian coccidiosis and necrotic enteritis (NE) are among the most economically significant diseases affecting the poultry industry worldwide.
- By using pyrosequencing technology, we have investigated the efficacy of dietary phytonutrient mixture, XT-6930® on the ileal microbiota of broiler chickens co-infected with *Eimeria maxima* and *Clostridium perfringens*.

## Materials and Methods

### ◆ Animal trial (Fig. 1)

- Birds: Cobb and Hubbard broiler chickens (75 each, one-day-old)
- NE induction:  $1.0 \times 10^4$  of *E. maxima* at 14 day post-hatch and  $1.0 \times 10^9$  CFU/ml of *C. perfringens* 4 days later.
- Experimental groups: assigned to 5 groups (15 birds/group): C (control), EM (*E. maxima* infected), CP (*C. perfringens* infected), NE (co-infected with EM and CP), XT (NE + XT-6930®).

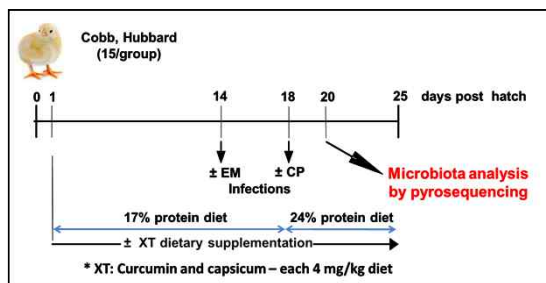


Figure 1. Schematic outline of the experimental design.

### ◆ Microbiota analysis

- Sampling: At 2 days post-CP infection, 5 birds from each group were killed and total genomic DNA extracted from ileal content.
- DNA extraction: UltraClean Fecal DNA Kit (MoBio, Carlsbad, CA)
- PCR amplification & pyrosequencing: barcoded primers targeting V1-V3 regions of 16S ribosomal RNA genes for the emulsion PCR with GS Junior system (Roche/454 Life Sciences)
- Pyrosequence analysis procedure: (Fig. 2)
- Taxonomic classification: each read was assigned against the EzTaxon-e database (<http://eztaxon-e.ezbiocloud.net>)

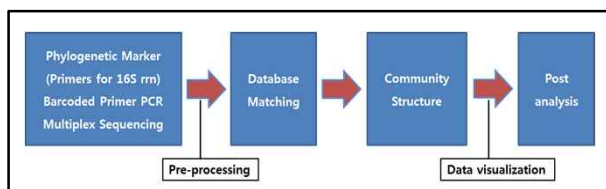


Figure 2. Pyrosequencing procedure used for the analysis of ileal microbiota.

## Results

- More than 60,000 partial 16S rDNA sequences obtained from 50 ileal samples were analyzed. Individual pyrosequencing reads corresponded to a specific operational taxonomic unit (OTU) and was assigned at the phylum, genus, and species level by homology comparison (Table 1).
- The number of reads per OTU allowed us to determine the relative abundance of each bacterial group comprising gastrointestinal microflora as well as the infected *E. maxima* strain for the challenge study.

## Results – cont'd

Table 1. Summary of the pyrosequence reads obtained from ileal microbiota of two different broiler chickens.

Breed	Treatment	Pyrosequence reads (No.)			Relative abundance of <i>Eimeria</i> (%)	No. of OTUs
		Total	Bacteria	<i>Eimeria</i>		
Cobb	C	6,361	6,361	0	0	23
	EM	9,021	8,799	44	0.5	20
	CP	8,530	8,530	0	0	27
	NE	1,056	956	100	9.4	15
	XT	9,905	9,505	400	4.0	162
Hubbard	C	3,733	3,733	0	0	43
	EM	10,660	10,335	325	3.1	22
	CP	3,218	3,218	0	0	10
	NE	6,264	5,071	1193	19.0	19
	XT	4,675	4,644	31	0.7	37

- Regardless of the breed of broiler chickens, pyrosequencing revealed the absence of *E. maxima* in their ileal contents from C and CP groups (Fig. 3).
- Relative abundance of *E. maxima* was significantly increased in NE group compared with EM group, suggesting that *C. perfringens* infection may have a potential role in *E. maxima* life cycle in their host (Fig. 3).
- Dietary supplementation with XT-6930® significantly decreased the relative abundance of *E. maxima* in their intestinal tract of both Cobb and Hubbard co-infected with EM and CP (Fig. 3 & 4).

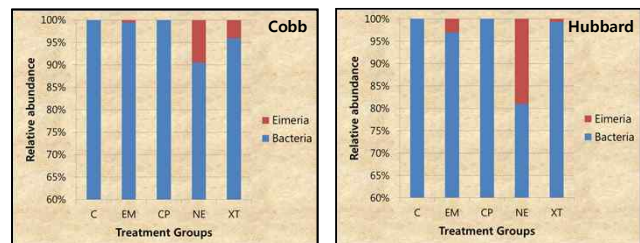


Figure 3. Changes of the relative abundance of *Eimeria* in the ileal microbiota in two chicken breeds. C: control; EM: *E. maxima* infected; CP: *C. perfringens* infected; NE: co-infected with EM and CP; XT: NE+XT6930®

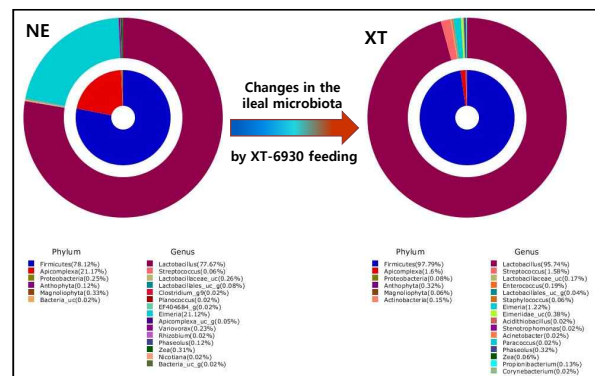


Figure 4. Comparison of the double pie charts generated from microbial communities in the ileum of Hubbard. Inner pie chart indicated the composition of phyla and outer pie chart represented the composition of genus. XT: NE+XT6930®

## Conclusions

- The results of this study show that dietary plant extracts change the composition of gut microbial communities and influence disease severity in avian coccidiosis and necrotic enteritis in commercial broiler chickens. The detailed mechanisms involved in this gut microbiota and immunity cross talk that lead to changes in disease phenotype is being investigated. Furthermore, we report for the first time a new method of enumerating *Eimeria* oocysts using pyrosequencing rather than manual counting.